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Original Research

Rapidly resorbable vs. non-resorbable suture for experimental colonic anastomoses in rats – A randomized experimental study

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ABSTRACT

Introduction: Anastomotic dehiscence remains an important challenge for colorectal surgeons worldwide. Extensive research focused on performing a safe anastomosis is conducted with rats being the most used model when examining colorectal anastomoses. In daily clinical practice resorbable sutures are used when hand-sewn anastomoses are performed. However, in the experimental studies examining colorectal anastomoses, non-resorbable sutures have predominantly been used. The aim of this study was to compare a rapidly resorbable suture with a non-resorbable suture in experimental colorectal anastomoses.

Methods: This was an experimental, prospective, case-control study using forty male Wistar rats. A colonic anastomosis was performed in a standardized fashion with either rapidly resorbable or non-resorbable suture. On the seventh postoperative day, the animals were sacrificed and the breaking strength of the anastomoses was measured.

Results: No suffering or poor wellbeing of the animals was registered. No animals died or were prematurely sacrificed. At tissue harvesting, no anastomotic leaks or signs of peritonitis were registered. The breaking strengths of the anastomoses were comparable in the two groups (median 2.175 (range 1.479–2.880) Newton vs. 2.267 (1.290–4.042) Newton ($P = 0.256$) for resorbable and non-resorbable sutures, respectively). We found no significant correlations between pre- to postoperative weight-loss and anastomotic strength.

Conclusion: Non-resorbable suture was comparable with rapidly resorbable suture with regards to breaking strength of an experimental colonic anastomosis. Thus, absorbable suture can be used in experimental studies which then more easily can be compared to clinical practice.

1. Introduction

With an overall anastomotic leakage rate in the range of 5–10% in colonic anastomoses,[1] anastomotic dehiscence remains an important challenge for colorectal surgeons worldwide. Due to this, there is extensive research focused on performing a safe anastomosis.[2–13] These studies are conducted mainly as experimental studies with rats being the most used model when examining colorectal anastomoses.[2–5,7,8,10–13]

In daily clinical practice human anastomoses are either stapled together or hand sewn. Resorbable sutures are used when hand-sewn anastomoses are performed. However, in the experimental studies examining colorectal anastomoses, non-resorbable sutures have predominantly been used.[2,4–9,11–13] The main disadvantage when using experimental studies is the difficulty of extrapolating results to clinical practice. Therefore experimental studies should mimic the normal clinical practice as much as possible.

The aim of this study was to compare a rapidly resorbable suture to a non-resorbable suture in experimental colorectal anastomoses to examine whether the method used in daily clinical practice can be used in experimental studies as well, and furthermore discuss the use of breaking strength as a model for anastomotic healing.

2. Methods

This was an experimental, prospective, case-control study. Forty male Wistar rats (Taconic, Ejby, Denmark) were used. The rats were divided equally into a Vicryl Rapide® or Ethilon® group. The investigators were blinded to the allocation until all data were analyzed. The study was performed at the Department for Experimental Medicine, the Panum Institute, Copenhagen, Denmark. Before the study period, the rats were acclimatized to the new surroundings for at least seven days. During the entire period the rats had free access to standard rat chow and tap water.

The animals were anesthetized using a combination of fentanyl and fluanisone (Hypnorm®, Janssen-Cilag EMEA, Beerse, Belgium) and midazolam (Dormicum®, F. Hoffmann-La Roche Ltd., Basel, Switzerland). A bolus of 0.3 ml/100 g was given as

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a subcutaneous injection. After 20 min, supplementary dosis of 0.15 ml/100 g Hypnorm–Dormicum was given. When anesthesia was achieved, the abdomen was shaved and sterilized using ethanol and chlorhexidine. The rats were then weighed, placed on an isolating flamingo-pad and covered with a sterile dressing.

The operative procedure was performed under aseptic conditions and by a single operator on the plateau faze of his learning curve (>70 experimental colonic anastomoses within three weeks). The abdomen was opened and a probe was inserted into the anus of the animal and led 6 cm up into the large intestine. This was done to ensure consistency in the level of the anastomosis. At the tip of the probe, which corresponds to the lower third of the descending colon, the intestine was divided sharply and a 1 cm resection of the colon was performed oral to the division site. Then, an anastomosis was performed with hand-sewn microsurgical technique using eight inverted single sutures. In one half of the animals, a rapidly absorbable 6–0 suture (Vicryl Rapide®, Ethicon Inc., New Brunswick, NJ, USA) and in the other half a monofilament non-absorbable 6–0 suture (Ethilon®, Ethicon Inc., New Brunswick, NJ, USA) was used. The anastomoses were performed while respecting the intestinal blood supply and were all tension-free and without distal obstruction. The abdominal muscle and fascia layers were closed using a continuous 3–0 monofilament non-absorbable suture (Ethilon®, Ethicon Inc., New Brunswick, NJ, USA). Finally, the skin incision was closed with titanium clips (Appose ULC, Covidien Healthcare Group, Norwalk, Connecticut, USA).

Before being placed in separate cages, the rats received 0.03 mg buprenorphine (Temgesic®, Schering-Plough Europe, Brussels, Belgium) as a subcutaneous injection for analgesia. For analgesia in the following three days, the animals were given 0.05 mg/kg buprenorphine mixed with chocolate cream (Nutella®, Ferrero Denmark, Herlev, Denmark) three times a day. The animal technicians recorded whether any animals were suffering or seemed ill in the postoperative period.

Seven days postoperatively, the animals were re-anesthetized using Hypnorm–Dormicum 0.3 ml/100 g. The abdominal cavity was opened, the anastomosis located and carefully freed of adhesions, resected en bloc and placed in isotonic saline at room temperature. The anastomotic breaking strength measurements were performed immediately (<2 min) hereafter. The animals were sacrificed immediately after harvesting the tissues for analyses.

The breaking strength of the anastomoses with the inverted sutures *in situ* were measured using a material testing machine (LF*Plus*, Lloyd Instruments, Fareham, UK) equipped with an XLC 10 N loading cell as described previously.[2] The tissue was placed between the clamps, which were 10 mm apart, and was pulled by a constant speed of 10 mm/min. The breaking strength was derived from the load-strain curve produced by the software (Nexygen®, Lloyd Instruments, Fareham, UK). Furthermore, it was registered if the break occurred in the anastomosis or in the adjacent healthy bowel and how the breaking occurred (sutures pulled through the tissue or breaking directly in the anastomotic line).

The study was approved from the Danish Council of Animal Experiments before initiation (J 2008/561–1583). According to the guidelines concerning humane endpoints, all animals that seemed to be suffering were immediately sacrificed. Data are presented as median (range). To analyze the results, Mann–Whitney's and Wilcoxon's tests were used to test for inter- and intragroup differences, respectively. For correlation analyses, Spearman's test was used. $P < 0.05$ was considered statistically significant. For statistical analyses, SPSS version 17 (SPSS Inc., Chicago, Illinois, USA) was used. Sample size calculation was performed based on anastomotic breaking strength measurements performed by Ågren et al.[2] where mean (SD) breaking strength was 1.90 (0.67) N on the seventh postoperative day. With alpha at 0.05, beta at 0.20 and minimally relevant difference at 35%, calculations showed that each group should comprise at least 16 animals. We chose to include a total of 20 animals in each group.

3. Results

During the entire study period, the animal technicians registered no suffering or poor wellbeing of the animals. Thus, no animals died or were prematurely sacrificed. At tissue harvesting, no anastomotic leaks or signs of peritonitis were registered.

The breaking strength of the anastomoses did not differ significantly in the two groups. In the Vicryl Rapide® group, median (range) breaking strength was 2.175 (1.479–2.880) Newton compared with 2.267 (1.290–4.042) Newton in the Ethilon® group ($P = 0.256$) (Fig. 1). For all the anastomoses, breaking occurred in the anastomosis and not in the adjacent bowel. It was evident, that breaking occurred when the sutures were pulled through the perianastomotic tissue.

The two groups were also comparable with regard to changes in body weight during the study. Median (range) weight-difference pre- to postoperatively was −12.5 (−57–13) g vs. −13.0 (−29–4) g in the Vicryl Rapide®- and Ethilon®-groups, respectively ($P = 0.797$).

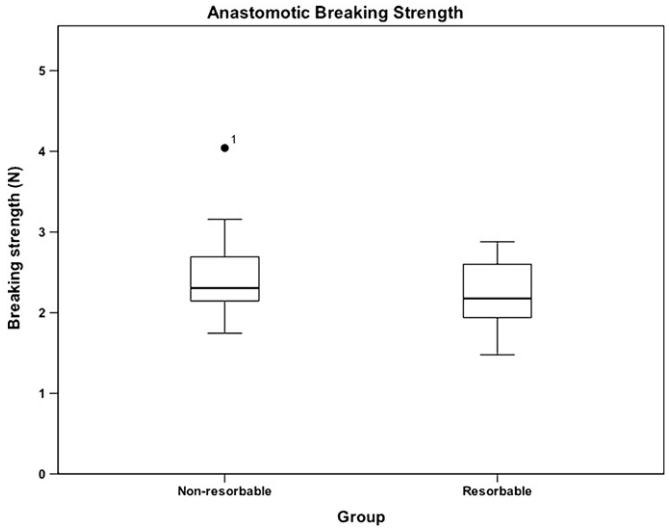

Fig. 1. Box-plot of anastomotic breaking strength. No significant differences between the groups.

We found no significant correlations between pre- to postoperative weight-loss and anastomotic strength. This applies for both pooled data (Spearman's rho = −0.018; $p = 0.26$) and for the two groups separately (non-resorbable: Spearman's rho = −0.26; $p = 0.27$, resorbable: Spearman's rho = −0.16; $p = 0.50$).

4. Discussion

In this experimental study we compared the breaking strength of colonic anastomoses in rats, sutured with either rapidly absorbable suture (Vicryl Rapide®) or non-resorbable (Ethilon®) suture, and we found no differences in the breaking strength measurements or any other of the registered parameters.

Hand-sewn colonic anastomoses in rats are often used for examining the effect of various interventions on colonic healing and anastomotic leakage.[2–4,7,10,13–15] In these studies either anastomotic breaking strength[2–4,13–15] or bursting pressure[7,10,14] have been used to evaluate anastomotic strength and thus serves as a surrogate marker for anastomotic leakage risk. The two methods of evaluating the anastomoses have been compared previously[11,12] and the two methods can both be used for assessing early healing of experimental colorectal anastomoses. Since the sutures are left *in situ* as the anastomoses are pulled apart, the breaking strength measurements used in this study provides information of the strength of the connective tissue surrounding the sutures. Thus, this measure has previously been named 'suture holding capacity'.[12] Tissue strength is influenced by total collagen content and the quality (i.e. mature/immature collagen ratio and number of cross-linkages) of the collagen.[16] It has previously been advocated that bursting pressure provides a more precise measurement of anastomotic strength[12] because distension is evenly distributed along the entire anastomotic line. However, since the force needed to disrupt the anastomosis depends on bowel radius, bursting wall tension (BWT) should be calculated when using bursting pressure to evaluate anastomotic strength, as described by Koruda et al.[16] Bowel radius is difficult to measure precisely and furthermore, the radius of adjacent bowel is normally larger than at the anastomosis and thus, bowel disruption tends to occur outside the anastomotic site.[17]

Non-resorbable monofilament sutures are normally preferred in experimental studies examining anastomotic strength because of reduced inflammation when compared to resorbable, braided

sutures and the risk of variable suture material degradation when using resorbable sutures.[18] Ågren et al. have described that the tissue surrounding the suture is subject to increased collagenolysis, histiocyte infiltration and matrix metalloproteinase activity leading to reduced matrix integrity[19] and this adverse reaction is possibly attenuated by the use of braided suture.[18] However, these suture materials are used in clinical practice and should therefore, if possible, also be used in experimental studies. In this study, we used the rapidly resorbable suture Vicryl Rapide®. The strength of this suture material is reduced to 50% after one week. In spite of this significant reduction we found no difference in anastomotic strength between the groups. This underlines the primary purpose of the sutures providing strength in only the immediate post-operative period.[20]

Few studies examining hand-sewn experimental colorectal anastomoses in rats have reported the number of anastomotic leakages. This fact elucidates an important limitation in these studies. It is difficult, even at autopsy, to decide objectively, if the animal has a leakage. This is due to extensive adhesion formation and fibrotic tissue, which can be hard to distinguish from infectious changes (abscess) and furthermore, the clinical effect of an eventual leakage and following peritoneal reaction is difficult to monitor and record.

In both groups, breaking occurred when the sutures were pulled through the perianastomotic tissue and not in the anastomotic line itself. Hence, although the strength of the Vicryl Rapide® sutures was reduced by 50%, the suture strength was greater than the suture holding capacity of the perianastomotic tissue. With the breaking occurring in a similar fashion in the two groups it is natural, that the breaking strengths registered were similar as well.

In conclusion, non-resorbable suture was comparable with rapidly resorbable suture with regards to breaking strength of an experimental colonic anastomosis. Thus, absorbable suture can be used in experimental studies which then more easily can be compared to clinical practice.

Ethical approval

The study was approved from the Danish Council of Animal Experiments before initiation (J 2008/561–1583).

Conflict of interest

No conflicts of interest.

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